Reference: *Biol. Bull* **187**: 309–318. (December, 1994)

Reproductive Potential and Genetics of Triploid Pacific Oysters, *Crassostrea gigas* (Thunberg)

XIMING GUO[1] AND STANDISH K. ALLEN, JR.

Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, B8, Port Norris, New Jersey 08349

Abstract. The reproductive potential and genetics of triploidy were studied in the Pacific oyster. DNA content in sperm from triploids showed a single peak at 1.5c as determined by flow cytometry. In eggs from triploids, trivalents were the dominant form of synapsed chromosomes, although the degree of synapsis varied considerably within and among females. Some eggs went through complete synapsis and formed 10 trivalent chromosomes; most had a mixture of 11–13 trivalents, bivalents, and univalents. Factorial matings were produced from diploid (D) and triploid (T) parent oysters, creating four crosses: DD, DT, TD, and TT (female first).

Gametes from triploids were fully capable of fertilization. After fertilization, eggs from triploids went through two meioses and released two polar bodies as diploid eggs did. Karyological analyses showed that average ploidy of the resultant embryos was 2.0 n for DD, 2.46 n for DT, 2.52 n for TD, and 2.88 n for TT. Survival of fertilized eggs to metamorphosis and settlement was about 21% for DD, but considerably lower on other crosses: 0.0007% for DT, 0.0463% for TD, and 0.0085% for TT. Nine months after matings, all survivors from DT crosses were diploid. Survivors from TD crosses consisted of 33% diploids, 57% triploids, and 10% tetraploids. Survivors from the TT crosses consisted of 90% triploids, 4% diploids, and 6% mosaics. We hypothesize that differences in ploidy composition between DT and TD embryos and survivors were caused by pro-egg segregations that favor the retention, rather than loss, of extra chromosomes in the egg. The reproductive potential of triploids and evolutionary implications are discussed.

Received 26 January 1994; accepted 22 September 1994.

[1] Corresponding author.

Introduction

Triploidy refers to the condition of a cell or organism having three sets of chromosomes. Such a condition in mammals is lethal. In many species of amphibians, fish, molluscs, and other invertebrates, triploids are fully viable and do not appear morphologically different from diploids. In a few species, such as certain gynogenetic fishes, triploidy is a natural mode of reproduction, but in most animals species, triploidy occurs infrequently and is considered a numerical mutation of chromosomes. However, triploidy can be easily induced in most lower animals by inhibiting the release of polar body II (Fankhauser, 1945; Thorgaard, 1983; Allen, 1986).

Triploids are commonly assumed to be sterile as a result of the extra set of chromosomes (Thorgaard, 1983). However, sterility in triploids is often incomplete and varies considerably among organisms. In plants, fertile triploids are common and are believed to have played a significant role in the evolution of plants (deWet, 1980). In salamanders, induced triploids were not completely sterile, and matings between triploids and diploids produced viable offspring (Fankhauser and Humphrey, 1950, 1954). In fish, induced triploids had severely reduced gonadal development in both sexes, although a small number of mature oocytes and sperm were observed (Lincoln, 1981a, b; Benfey and Sutterlin, 1984; Benfey, 1991; Gui *et al.*, 1991). No viable fish were obtained when triploid males were crossed with diploid females (Lincoln, 1981a; Lincoln and Scott, 1984). In invertebrates, triploid *Drosophila* produced viable offspring when mated with diploids (Morgan, 1925). In molluscs, triploids have been produced in a number of species, but so far most studies have been limited to the induction, growth, and gonadal development of triploids (Beaumont and Fairbrother, 1991). Although triploid molluscs generally show retarded gonadal

development, the formation of apparently normal gametes was observed in both sexes of triploid Pacific oyster, *Crassostrea gigas* (Allen and Downing, 1990; Guo, 1991) and several other species. In the Pacific oyster, Allen (1987) made crosses between triploid males and diploid females and obtained viable offspring.

The reproductive potential and genetics of triploids is relevant to our understanding of animal evolution, particularly the increased attention on the evolutionary significance of polyploid animals (White, 1978; Schultz, 1980; Bogart, 1980). If triploidy occurs spontaneously in most species, what are the genetic consequences of triploidy? Is triploidy simply a genetic burden on the population, or is it a mutation that has vital importance for the evolutionary process? Our present knowledge about the biology and reproductive genetics of polyploid animals is limited, especially among marine invertebrates.

The reproductive potential and genetics of triploidy also has important practical implications. In the Pacific oyster, for example, triploids have been produced commercially for aquaculture since 1986 (Allen *et al.*, 1989). What impact may triploids have on the genetic structure of natural populations when released? Triploids have also been proposed as candidates for the testing of non-native species (Allen, 1993). If triploids are not completely sterile, are they likely to become established? This study was conducted to examine the reproductive potential and genetics of triploidy using the Pacific oyster as a model species.

Materials and Methods

To study the reproductive genetics of triploid Pacific oyster, matings were conducted within/between diploids (D) and triploids (T), producing four crosses: DD, DT, TD, and TT, with the female listed first. Triploids used in this study were two years old and had been induced by inhibiting the release of the second polar body in fertilized eggs (Allen *et al.*, 1989). Before making crosses, triploids were individually confirmed by flow cytometry. Gametes were obtained by dissecting gonads. Eggs were passed through an 85-μm screen to remove the large tissue debris and then rinsed on a 25-μm screen. Sperm was passed through a 15-μm screen to remove tissue debris. Fertilization and incubation were conducted at 25°C using filtered (2 μm) seawater. Embryos were cultured according to routine hatchery protocol (*e.g.*, Breese and Malouf, 1975).

Synapsis and meiotic segregation in eggs from triploids was analyzed in TD crosses. In all crosses, the ploidy of two-hour-old embryos was determined by chromosome counts, and the ploidy of 24-hour-old embryos and later survivors was determined by flow cytometry. Survival to D-stage (Day 1), Day 7, and Spat (Day 30) was recorded in each group. Each cross was repeated four to seven times using different pairs of oysters as parents.

Synapsis, meiotic segregation, and chromosome constitution were observed by aceto-orcein stain (Guo *et al.*, 1992). Briefly, eggs were fixed in acetic acid and methanol (1:3). A drop of fixed sample was loaded on a slide, mixed with 2–3 drops of orcein stain (0.5% orcein in 60% acetic acid), and covered with a cover glass. After staining for five minutes, the eggs were gently compressed by applying pressure to the cover glass, which was then sealed. For chromosome counts, 2–4 cell embryos were first treated with 0.005% colchicine for 15 min before they were fixed. Flow cytometry was conducted on a Partec II flow cytometer with a 4,6-diamidine-2-phenylindole (DAPI) stain (Allen, 1983; Guo *et al.*, 1993).

To make sure survivors were not contaminants, allozyme inheritance was analyzed with starch gel electrophoresis (Aebersold *et al.*, 1987; Hebert and Beaton, 1989). Allozymes examined included aspartate aminotransferase (AAT, EC 2.6.1.1.), dipeptidase (DAP, EC 3.4.-.-, Gly-leu as substrate), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), glucose phosphate isomerase (GPI, EC 5.3.1.9), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), mannose phosphate isomerase (MPI, EC 5.3.1.8), phosphoglucomutase (PGM, EC 2.7.5.1), superoxide dismutase (SOD, EC 1.15.1.1), tripeptidase (TAP, EC 3.4.-.-, Leu-gly-gly as substrate).

Results

Gametes from triploids

Seventy-seven triploid oysters were examined during this study, and gametes were observed in 60 (78%) of them. Based on the type of gametes observed, the 60 oysters consisted of 35 females (58%), 16 males (27%), and 9 hermaphrodites (15%). Nineteen of the triploid females that produced a large number of eggs were used for crosses.

Twenty-two crosses were made: six DD, seven DT, five TD, and four TT. Most of those crosses were not complete 2×2 factorials between diploids and triploids due to limitations of triploid parents. When only triploid females (or males) were found in a given day, they were used to make TD (or DT) crosses with DD controls. Later in the study, the lack of TT crosses was apparent, and seven TT crosses were made on the same day and combined into one mass culture.

Among the 19 females used in crosses, the number of eggs ranged from 19 thousand to 21.5 million, with a mean of 2.3 million and a median of about 1 million (Table 1). The numbers of eggs in the other 16 triploid females were deemed too few for making crosses and were not determined, but they were probably fewer than 19,000. Assuming that the 16 females not counted averaged about 15,000 eggs, the estimated fecundity of triploid females would be around 1.2 million eggs per female. Eggs in two

Table I

Fecundity of two-year-old triploid females of the Pacific oyster (median value in bold)

Female	Number of eggs ($\times 10^3$)
1	19.0
2	23.8
3	28.6
4	57.2
5	240.5
6	313.5
7	339.0
8	454.5
9	699.6
10	**968.0**
11	1,420.4
12	1,425.0
13	1,755.6
14	1,782.0
15	2,145.0
16	2,398.0
17	3,045.0
18	4,325.0
19	21,520.0
Mean	2,261.0

2-year-old diploids were counted during this study, and the egg numbers were 25 and 105 million per female, agreeing with the fecundity estimates of Quayle (1988) of 50–100 million. We conservatively estimate 50 million eggs as the average fecundity for diploid females of this age. Therefore, this study suggests that the relative fecundity of triploid females is about 2% of normal diploids.

Diameters of 10 eggs were measured for four diploids and six triploids. Within females there was little variation in egg diameter from both diploids and triploids, the coefficient of variation ranging from 1.8 to 3.3%. On the other hand, variation among diploid females and among triploid females was highly significant (ANOVA, F-test, $P < 0.0001$ for both diploids and triploids). Eggs from all triploids were significantly larger than those from diploids ($P < 0.0001$). Eggs from diploids had an average diameter of 53.0 μm, ranging from 50 to 55 μm. Eggs from triploids had an average diameter of 58.2 μm, with a range of 56 to 62 μm. On average, the diameter of eggs from triploids was 10% larger than eggs from diploids, which corresponds to a 33% increase in cell volume.

In the eggs, synapsed chromosomes became visible after germinal vesicle breakdown (15 to 30 min in 25°C seawater). Eggs from diploids had exactly 10 bivalents (synapsed homologous chromosomes) (Fig. 1A), with no detectable variation within or between females (Table II). On the contrary, synapsis in eggs from triploids was highly variable and chaotic. In some eggs, synapsis was complete with the formation of 10 trivalents (Fig. 1B). In others, synapsis was incomplete, showing a mixture of tri-, bi-, and univalents (Fig. 1C). Overall, trivalents were the dominant form of synapsed chromosomes. A few eggs exhibited "over-synapsis" and formed eight or nine chromosomes containing multivalents greater than three.

A precise count of valent formations was not possible, but total numbers of chromosomes (all forms) were determined. Among all eggs examined from triploids, the number of chromosomes ranged from 8 to 18, with an overall mean of 12. The average chromosome number among triploid females varied from 10.6 and 13.9 (Table II). Within females, chromosome number also varied considerably (Fig. 2). Of nine triploid females analyzed, two females had a modal chromosome number of 10 per egg (*e.g.*, Fig. 2, F1); three females had a modal chromosome number of 11 (*e.g.*, Fig. 2, F2); four had a modal number of 13 (*e.g.*, Fig. 2, F3). There was no correlation between the completeness of synapsis and fecundity ($r = 0.17$, $P = 0.76$, $n = 6$).

When analyzed by flow cytometry, sperm from triploid males had an average DNA content of 1.49c (1c = 1n = 10 chromosomes) (Table III). No haploid peaks were observed among triploid males in this study.

Fertilization and egg development

Gametes from triploids were fully capable of fertilization. On average, fertilization among crosses with triploid parents was slightly lower than that of DD crosses (Table IV). However, the range in all groups was approximately the same, suggesting that gametes from triploids had full potential for successful fertilization.

After fertilization, the development of eggs from triploids was examined in TD crosses. Like normal eggs, eggs from triploids resumed meiosis by gathering chromosomes within minutes post-fertilization (PF). Polar body I (PB1) was released in the majority of the eggs between 10 and 20 min PF. After PB1 release, meiosis in eggs from triploids continued with, on the average, 15 bivalents at metaphase (Fig. 1D). Anaphase II was reached in the majority of the eggs between 35 and 45 min PF (Fig. 1E). Polar body II (PB2) was released in the majority of the eggs by 50 min PF. In TD crosses, highly elongated maternal (15 on the average) and paternal (always 10) chromosomes reappeared in two separate groups around 60 min PF. They mixed and condensed into typical metaphase-shaped chromosomes for the first mitotic division (Fig. 1F). In some crosses, polar body releases and early cleavages among eggs from triploids were slightly slower and less synchronized than those in eggs from diploids. At 2 h PF, the majority of the TD embryos entered the 4-cell stage.

Chromosome numbers were determined for embryos in one DD, three DT, three TD, and four TT crosses.

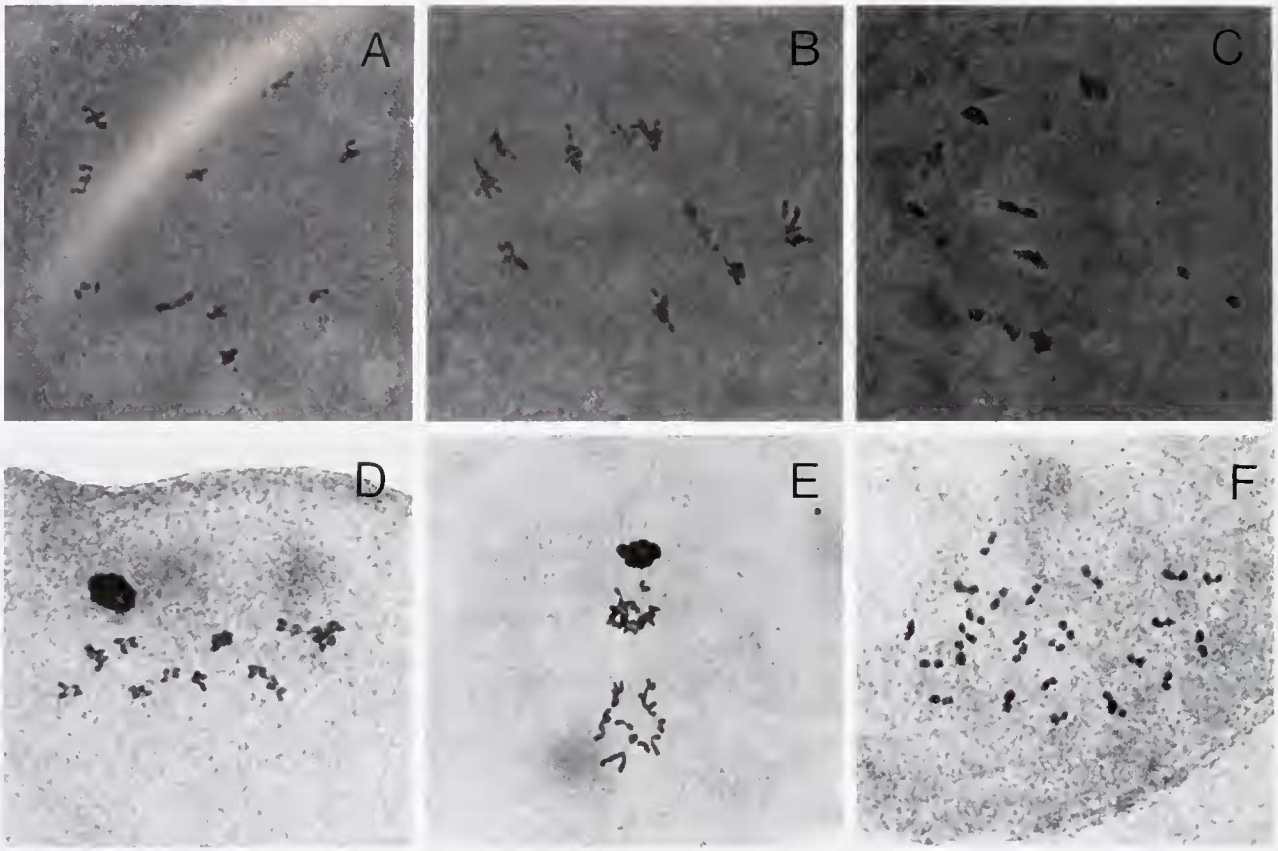

Figure 1. Synapsis and meiosis in eggs from triploid Pacific oysters: A—metaphase I eggs from diploids with 10 bivalents; B—complete synapsis in metaphase I eggs from triploids with 10 trivalents; C—incomplete synapsis in metaphase I eggs from triploids with a mixture of uni-, bi-, and trivalents; D—metaphase II in eggs from triploids; E—anaphase II in eggs from triploids; and F—metaphase of the first mitotic division of an embryo from a triploid (female) × diploid cross.

The orcein staining protocol used in this study was highly reliable for determining chromosome numbers in early embryos. Often more than half of the embryos had metaphases that could be clearly counted, and it was not unusual for embryos to have two countable metaphases. Most importantly, there is no artifactual chromosome loss. In the DD crosses, 49 of the 50 embryos analyzed had 20 chromosomes, and one had 30 chromosomes (Table V). In other crosses, the average chromosome numbers were 24.6 for DT, 25.2 for TD, and 28.8 for TT. TD embryos had a slightly greater mean chromosome number and coefficient of variation than DT crosses. The distribution of chromosome numbers among embryos in DT and TD crosses is shown in Figure 3. Comparatively, the distribution of chromosome numbers of DT embryos was closer to that expected from the random segregation of the third chromosome set than was the distribution of chromosome numbers in TD embryos. The latter had more embryos with 25+ chromosomes than DT crosses. Flow cytometric analysis of 24-hour-old embryos showed the same pattern, and the mean ploidy levels were 2.45 c for DT, 2.46 c for TD, and 2.90 c for TT crosses (Table III). Mean coefficient of variation of DNA content among the four groups was highest in the TD cross (Table III). Over time, the aneuploid peak in TD crosses split into two peaks: one close to 2 n and the other close to 3 n (Fig. 4). The peak splitting suggests that aneuploids closest to euploidy survive longer.

Survival

Survival of the fertilized eggs to D-stage was 65.8% for DD, 48.9% for DT, 31.7% for TD, and 39.5% for TT crosses (Table IV). Survival of fertilized eggs to Day 7 in crosses with triploid parents (0.50–7.9%) was obviously lower than that in DD crosses (39.8%). Surprisingly, TD crosses (7.9%) had much higher survival to Day 7 than DT crosses (0.52%). The same pattern of survival continued to 2 months PF: 20.6% for DD, 0.0007% for DT, 0.0463% for TD, and 0.0085 for TT crosses. When stan-

Table II

Total number of chromosomes (uni- and multivalents) in eggs from diploid and triploid Pacific oyster before fertilization

	Female	n	Mean	Difference*
2n	1	10	10.0	a
	2	15	10.0	a
			10.0	
3n	1	16	10.6	ab
	2	15	10.6	ab
	3	48	10.8	abc
	4	19	11.5	abcd
	5	12	12.2	bcde
	6	12	12.9	de
	7	44	13.2	e
	8	20	13.4	e
	9	10	13.9	e
			12.0	

* Different letters designate significant differences in means among females, as indicated by the Tukey HSD multiple comparison at a confidence level of 95%.

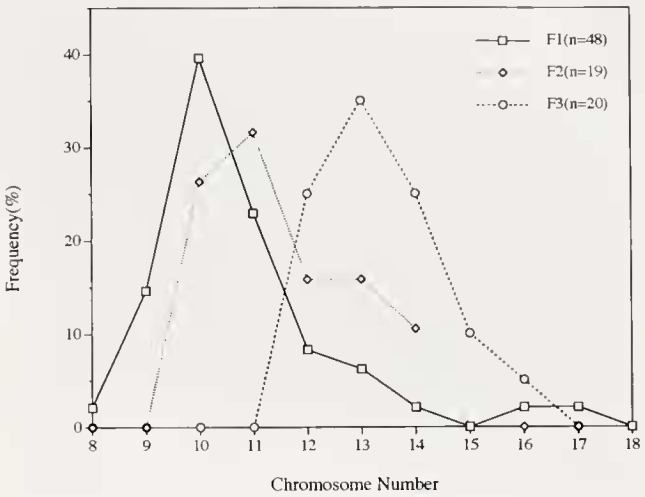

Figure 2. Distribution of chromosome numbers (uni, bi, tri, and multivalents) in Pacific oyster eggs from three different triploid females (F1–F3) prior to fertilization.

dardized by the survival of DD crosses, the survival of triploid crosses to two months was 0.0034% for DT, 0.2248% for TD, and 0.0413% for TT crosses.

At nine months PF, all experimental oysters died from an accidental crash of our rearing system. Tissues of some of the newly deceased oysters were saved, and their ploidy levels were analyzed by flow cytometry. All 30 oysters from one of the DD crosses were diploids. Twelve oysters from one of the DT crosses were also diploid. On the contrary, only one-third (32.5%) of the 40 TD oysters examined were diploid, and the other two-thirds were either triploid (57%) or tetraploid (10%). In the TT mass cultures which escaped the accidental crash, 48 oysters lived to one year of age. As analyzed by flow cytometry, the surviving TT oysters consisted of 90% triploids, 4% diploids, and 6% 2 n/3 n mosaics.

Allozyme patterns in DT survivors (all diploid) and all parents used in this study were analyzed. Out of the 11 allozymes analyzed, eight were successfully resolved, and five were polymorphic. No foreign alleles were detected, and we conclude survivors were not contaminants. Survivors from TD and TT groups were not analyzed, because the survivors were primarily polyploids that could not have been contaminants. However, we cannot rule out that the diploids found in either TD or TT crosses were the result of contamination from another stock or culture.

Discussion

Meiosis in triploids

Trivalents were clearly the predominant form of synapsed chromosomes in eggs from triploid Pacific oysters, although incomplete synapsis was frequent with a mixture of tri-, bi-, and univalents. The formation of multivalents is common in polyploid plants and animals and has often been used as an indicator of recent polyploidization. Among 14 autotetraploid plants, for example, quadrivalent formation averaged 45%, ranging from 4 to 81% (Cauderon, 1986).

Completeness of synapsis varied considerably among females—variation that we cannot explain. Notably, there was no correlation between the degree of synapsis and fecundity, although our sample size was small (n = 6); further studies are needed to verify this observation. If

Table III

Relative DNA content measured by flow cytometry in parents and progeny of crosses within/between diploid (D) and triploid (T) Pacific oysters

Tissue	Group	n	Mean	SE	CV
Parent, somatic	2n	15	1.95	0.03	—
	3n	14	2.92	0.03	—
Parent, sperm	2n	15	1.00	*	—
	3n	11	1.49	0.01	—
Progeny, 24 h	DD	6	2.00	*	1.80
	DT	6	2.45	0.04	2.44
	TD	6	2.46	0.05	4.12
	TT	5	2.90	0.05	3.55

The first letter in the progeny group refers to the ploidy of the female. SE is standard error of mean DNA content in observations. CV is mean coefficient of variation of DNA histograms, where CV = 100* (standard deviation/mean).

* Used as standards.

Table IV

Percent survival of fertilized eggs to D-stage, to 7 days post fertilization (PF), and to spat (3 months PF) in crosses within/between diploid (D) and triploid (T) Pacific oyster; the first letter in the cross label refers to the female ploidy

Cross	Eggs ($\times 10^3$)	Fertilization (%)	D-stage (%)	7 days (%)	Spat (%)
DD					
1	1,811	61.5	84.7	—	—
2	381	95.2	97.8	32.2	18.7
3	103	9.6	22.2	19.5	—
5	1,574	50.3	40.6	40.6	27.1
8	1,400	94.9	63.9	43.5	23.3
9	1,748	94.6	85.6	63.4	13.3
		67.7	65.8	39.8	20.6
DT					
1	1,573	23.8	67.1	—	0.0043
3	1,027	13.7	100.0	0.13	—
4a	551	75.7	24.1	0.90	0
4b	103	77.9	18.0	1.27	0
5	3,148	40.9	26.9	0.10	0.0001
8	2,700	82.3	26.9	0.10	0
9	1,869	92.8	79.0	0.61	0
		58.2	48.9	0.52	0.0007
TD					
2b	622	33.6	26.2	9.14	0.0325
2c	149	66.7	17.5	4.45	0.1180
5a	996	16.9	28.0	19.30	0.0178
5b	115	20.7	29.8	6.20	0.0628
9	1,234	95.6	57.1	0.54	0.0005
		46.7	31.7	7.93	0.0463
TT					
6	878	9.0	40.8	0.40	0
7	11,974	—	16.1	0.83	0.0337
9a	1,164	89.3	80.4	0.67	0.0004
9b	700	31.8	20.7	0.11	0
		43.4	39.5	0.50	0.0085

fecundity is unrelated to completeness of synapsis in triploids, their low fecundity must be caused by factors other than synapsis, contrary to common belief. The inability to complete meiotic synapsis has been suggested as the cause for triploid sterility (or retarded gonadal development) in several species of fish (Thorgaard and Gall, 1979; Thorgaard, 1983, 1986; Benfey *et al.*, 1986). It is also difficult to argue that the low fecundity of triploid females is caused by aberrations in trivalent segregation. First, meiotic divisions remain arrested until after fertilization (Lu, 1986; Guo *et al.*, 1992). Second, triploid eggs seemed to complete meiosis normally.

For the most part, the extra set of chromosomes in triploids segregated randomly during meiosis. The sperm from triploid males had an average DNA content of 1.5 c, and no haploid sperm peaks were observed by flow cytometry. Allen (1987) observed that some triploid males produced haploid sperm; it is uncertain whether mosaic (2 n/3 n) triploids were involved. The random segregation of the extra chromosome set during meiosis was confirmed also by the fact that mean chromosome number of 2–4 cell embryos in both DT and TD embryos was about 2.5 n. There are no comparable studies on the ploidy of 2–4 cell embryos. Chromosome data in amphibians and plants were collected at late larvae or young plant stages and are therefore biased because of the likely differential mortality of aneuploids (Myers, 1944; Punyasingh, 1947; Fankhauser and Humphrey, 1950, 1954). On the other hand, the ploidy composition of survivors observed in this study agrees with findings from previous studies. In both plants and amphibians, polyploids were frequently observed from TD survivors, but rarely from DT survivors (Jones and Bamford, 1942; Fankhauser and Humphrey, 1950, 1954). In the Pacific oyster, the only study on DT crosses found that 149 of the 150 survivors analyzed were diploids (Allen, 1987), which supports our data.

Table V

Chromosome number in 2–4 cell embryos in experimental crosses within/between diploid (D) and triploid (T) Pacific oysters

Cross	n	Mean	CV
DD			
1	50	20.2*	7.0*
DT			
1	30	24.4	7.3
2	50	24.5	5.0
3	50	24.7	5.0
		24.6	5.8
TD			
1	17	25.0	7.6
2	46	25.0	10.2
3	71	25.5	11.6
		25.2	9.8
TT			
1	50	28.6	5.5
2	51	28.7	6.9
3	34	29.1	7.7
4	33	28.9	6.3
		28.8	6.6

CV = coefficient of variation 100* (standard deviation/mean chromosome count). The first letter in the cross label refers to the female ploidy.

* Mean > 20 and variance due to a single triploid. Otherwise DD mean = 20, CV = 0.0.

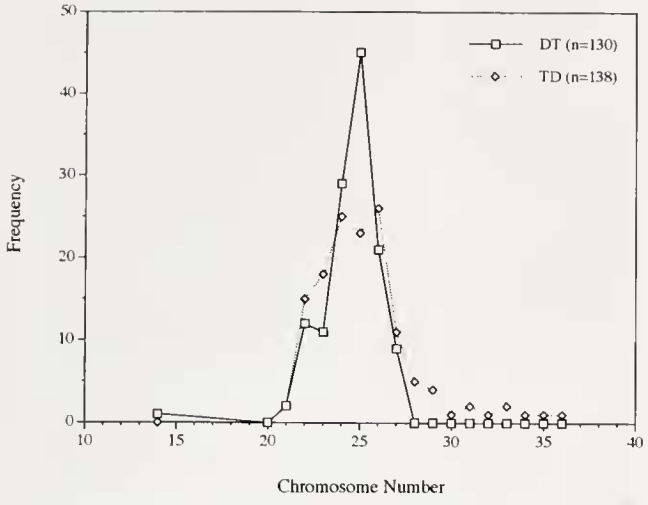

Figure 3. Distribution of number of chromosomes per cell in 2–4 cell diploid (female) × triploid (DT) and triploid (female) × diploid embryos (TD) of the Pacific oyster, showing some TD embryos had high chromosome numbers where DT did not.

Results of this study suggest that there are important differences between meiosis in triploid males and females. First, chromosome numbers in DT embryos are closer to the theoretical distribution than those in TD embryos, and TD embryos had more cells with 25+ chromosomes than DT embryos. Second, survivors from TD crosses were primarily polyploid, and survivors in DT crosses were primarily diploids. These observations suggest that meiosis in triploid females is more likely to make mistakes and produce gametes with chromosome gains rather than losses, compared with meiosis in triploid males. We hypothesize that in eggs from triploids, there is a general mechanism of *pro-egg segregation* which favors the retention, rather than loss, of chromosomes by the egg pronucleus.

In eggs, meiotic divisions are characterized by the formation of two polar bodies. We propose that segregation errors occur more often in the egg than in sperm; and when those errors occur, the egg tends to retain the errant chromosome(s) (either univalents or nondisjoined bivalents) rather than rejecting them into the polar body. In contrast, meiotic divisions in males produce four sperm, and therefore missegregated chromosomes are randomly distributed into daughter cells by default. The suppression of meiosis I and the formation of diploid gametes can be considered extreme cases of pro-egg segregation, which rarely occur in males. Pro-egg segregation would have a selective advantage because the gain of chromosomes is generally less harmful than their loss. Overall, the pro-egg segregation hypothesis predicts that meiotic segregation is random and more accurate in males than in females, and when missegregation occurs in the egg, there is a tendency for the egg to gain chromosomes rather than lose them.

Pro-egg segregation provides one explanation for the differences in DT and TD crosses observed in this study, and may be relevant to cytogenetic abnormalities in other taxa, such as human aneuploidy. Aneuploidy is a frequent form of chromosome abnormality in humans. There are two characteristics associated with human aneuploidy. First, there is a prevalence of trisomics (hyperploidy) over monosomics (hypoploidy) among spontaneous abortions and liveborns (Hecht and Hecht, 1987). Second, 90–95% of the trisomics receives the extra chromosome from the mother (Takaesu *et al.*, 1990; Antonarakis *et al.*, 1991,

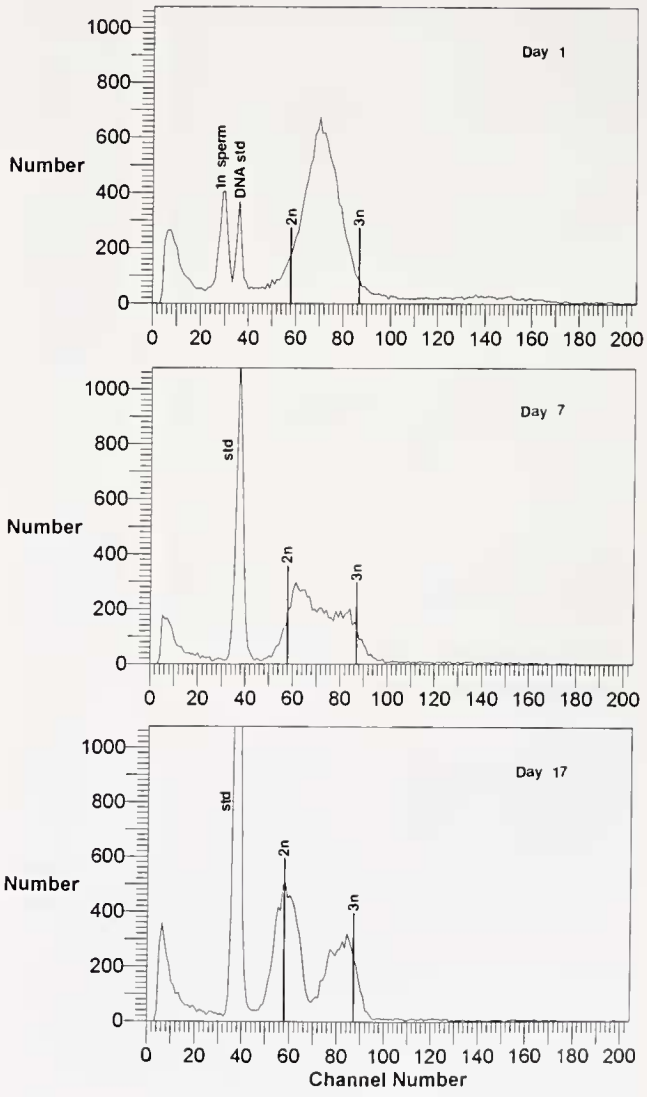

Figure 4. Relative DNA content measured by flow cytometry of dissociated cells of triploid (female) × diploid (TD) embryos of the Pacific oyster 24 h (top) and 17 days (bottom) days post fertilization, showing the loss of aneuploids and survival of larvae closer to euploid DNA contents of 2 n or 3 n.

1992), and there is a positive correlation between the frequency of trisomics and maternal age (Hecht and Hecht, 1987). The pro-egg segregation provides a possible explanation for these characteristics. An alternative explanation for the prevalence of trisomics among spontaneous abortions is the possibility that all monosomics die at an early stage before the recognition of pregnancy. Direct comparison of hypo-/hyperploidy ratio in mammalian sperm and oocytes has not been possible, because of the artifactual nature of hypoploid data. Unlike the orcein stain method used in this study, the standard air-dry procedure in mammalian cytogenetics is prone to chromosome loss. In fact, cytogenetic analyses in mammals often discard hypoploid data completely, and estimate the total aneuploidy frequency by doubling the hyperploidy frequency (Mailhes, 1987; Pellestor, 1991; Zenzes and Casper, 1992). (The assumption of hypo- and hyperploidy occurring at the same frequency would be invalid under the pro-egg segregation hypothesis.) Nevertheless, it has been well recognized that meiotic differences exist between human males and females. One of the hypotheses attributes the excessive non-disjunction in oocytes to the compromised microcirculation (Gaulden, 1992). Another hypothesis views that non-disjunction occurs randomly at all chromosome pairs in males, but not in females (Hecht and Hecht, 1987; Pellestor, 1991). Others have primarily focused on the positive correlation between trisomics and maternal age (Warburton, 1989; Eichenlaub-Ritter and Boll, 1989; Gaulden, 1989, 1992; Kloss and Nesse, 1992; Kratzer *et al.*, 1992; Zheng and Byers, 1992). The only one that is similar to the pro-egg segregation hypothesis is the chromosome competition hypothesis by Axelrod and Hamilton (1981). Recognizing the excess of hyperploids and their positive correlation with maternal age, Axelrod and Hamilton suggested that hyperploidy was due to competition among chromosomes to get into the oocyte rather than the polar body, as the mother approaches menopause.

There may be other mechanisms responsible for the apparent differences in meiosis between triploid females and males in this study. The formation of tetraploids in TD crosses, for example, could be caused by spontaneous suppression of meiosis I (Wilson, 1946; Fankhauser and Humphrey, 1950). In the Pacific oyster, blocking polar body I in TD zygotes indeed produced high proportions of tetraploids (Guo and Allen, 1994a). However, suppression of meiosis I, or even meiosis II, cannot explain the formation of a high proportion of diploid gametes in triploid females (corresponding to 3 n TD survivors).

Reproductive potential of triploids

This study clearly shows that triploid Pacific oysters are not completely sterile. Despite the fact that the majority of progeny from triploids were aneuploids, survivors were obtained from all crosses. This study also demonstrates that the majority of Pacific oyster aneuploids with intermediate chromosome numbers are inviable. Survivors that were classified as euploids in this study actually may be aneuploids because flow cytometry cannot detect small differences in DNA content without rigorous standardization. Viability of trisomics, hyper-triploids, hypo-, and hypertetraploids was demonstrated previously in the Pacific oyster (Guo and Allen, 1994a).

Because gametes from triploids were fully capable of fertilization, the reproductive potential of triploids primarily depends on their fecundity and survival of offspring. The relative fecundity of the triploid females was about 2% of diploid. Unfortunately, sperm production in triploid males was not determined in this study. Our observations seem to suggest that the relative fecundity (relative to diploids) of triploid males might be even lower than triploid females. Comparable fecundity data are not available in the literature, and most studies on the sterility of triploid animals are limited to histological observation of gonadal development (Beaumont and Fairbrother, 1991). In salamanders, triploid females produced half as many eggs as diploids (Fankhauser and Humphrey, 1950). In the clam (*Mulinia lateralis* Say), the relative fecundity of triploid females and males was about 59% and 80%, respectively, compared with diploids (Guo and Allen, 1994b). Among 500 triploids examined in the carp (*Carassius auratus*), one female was found to have a normally developed ovary filled with normal eggs (Gui *et al.*, 1991).

The results of our TT crosses are relevant to population control, as in the case where pure triploid populations are used in the culture or testing of a non-native species. Assuming that sperm is not the limiting factor (a conservative concession), the reproductive potential may be estimated as the product of reduced fecundity of triploids (0.02 of diploids) and survival of TT progeny relative to diploids (0.0085%/20.6%), or about 0.0008% of normal diploids in the first generation. For example, when diploids have 1 million chances to reproduce, triploids would have about 8. Furthermore 90% of TT survivors were triploid, although the chance of producing a diploid is not zero.

The other situation is a population mixed with diploids and triploids, as in the case where triploids (*e.g.*, Pacific oysters) are cultured in close proximity to a normal diploid population. Here, the fecundity of triploid males needs to be considered also. Assuming the triploid males also had a relative fecundity of 2%, triploids would have a reproductive potential of 0.0046% of normal diploids, *i.e.*, 46 in one million. The sperm production in triploid males may not be 2%, but since reproductive potential is primarily due to survival of TD, and not DT, crosses, our estimate would change little. In subsequent generations, the involvement of tetraploids—if fertile—may favor the

production of triploids. Reproductive potential of tetraploids needs to be examined further.

These estimates of reproductive likelihood are preliminary, and many factors could affect the reproductive potential of triploids. For example, environmental factors could affect fecundity of triploids. We noticed that triploids from Washington State produced very few eggs, compared with ours reared in quarantine in New Jersey. More importantly, the reproductive potential estimated in laboratories probably overestimates that from natural situations. However, given adequate study, the reproductive potential of triploids is quantifiable.

Evolutionary implications

Reproduction in triploid Pacific oysters may have evolutionary implications. In plants, polyploid evolution has been described as a two-step process (deWet, 1980). The first step is the formation of triploids (often during hybridization); the second step—production of tetraploids from crosses between triploid females (3 n gametes) and diploid males (1 n gametes). Fankhauser and Humphrey (1950) duplicated these steps in the laboratory with the salamander, but their findings were largely neglected by evolutionary zoologists. Our finding of the same process in the Pacific oyster suggests that the triploid female × diploid male scenario may be a general phenomenon occurring in other animal groups. In special cases of fish and amphibians, the transition from triploid to tetraploid is accomplished by an endomitosis before meiosis (Schultz, 1980). However, endomitosis is rare and primarily limited to a group of all-female, gynogenetic fish (Cimino, 1973). Our data, along with those of Fankhauser and Humphrey (1950) plus data from plants, suggest that the triploid female × diploid male scenario may serve as a general mechanism for evolution by polyploidy in both animals and plants.

Another interesting finding of this study is that crosses within triploids produce primarily triploids, proving that triploids have some fertility and apparently can reproduce themselves genetically. The possibility of triploids mating with triploids exists in a hybridization zone where triploid hybrids are more viable than diploid hybrids. Those triploid hybrids may potentially mate with each other to reproduce themselves, or mate with diploids to produce fertile tetraploids. It has been documented in several species of fish that hybridization is only viable as triploids (Chevassus *et al.*, 1983; Scheerer and Thorgaard, 1983; Arai, 1984, 1986; Parson *et al.*, 1986; Yamano *et al.*, 1988).

Acknowledgments

We are grateful to Greg A. DeBrosse for care of brood stock, larval, and nursery cultures, and to Ann E. Arseniu for general lab assistance. This is New Jersey Agricultural Station Publication No. D-32100-1-94 and Contribution #94-03 of the Institute of Marine and Coastal Sciences, Rutgers University, supported by NOAA grant NA16FL0409-01 (91-ODR) to SKA and USDA/CSRS/OGPS 92-3703-7766 to SKA and XG.

Literature Cited

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. *Manual for Starch Gel Electrophoresis: A Method for the Detection of Genetic Variation.* NOAA Technical Report NMFS 61, USA.

Allen, S. K., Jr. 1983. Flow cytometry: assaying experimental polyploid fish and shellfish. *Aquaculture* **33:** 317–328.

Allen, S. K., Jr. 1986. Genetic manipulations: critical review of methods and performances in shellfish. Pp. 127–143 in *Selection, Hybridization and Genetic Engineering in Aquaculture, Vol. II,* K. Tiews, ed. Heenemann Verlag, Berlin.

Allen, S. K., Jr. 1987. *Reproductive Sterility of Triploid Shellfish and Fish.* Ph.D. dissertation, University of Washington, Seattle, Washington.

Allen, S. K., Jr. 1993. Triploids for field study? The good, the bad, and the ugly. *J. Shellfish Res.* **12:** 125 (abstract).

Allen, S. K., Jr., and S. L. Downing. 1990. Performance of triploid Pacific oysters, *Crassostrea gigas.* Gametogenesis. *Can. J. Fish. Aquat. Sci.* **47:** 1213–1222.

Allen, S. K., Jr., S. L. Downing, and K. K. Chew. 1989. *Hatchery Manual for Producing Triploid Oysters.* University of Washington Press, Seattle, Washington.

Antonarakis, S. E., and the Down Syndrome Collaborative Group. 1991. Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphism. *N. Engl. J. Med* **324:** 872–876.

Antonarakis, S. E., M. B. Petersen, M. G. McInnis, and P. A. Adelsberger. 1992. The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphisms. *Am. J. Hum. Genet.* **50:** 544–550.

Arai, K. 1984. Developmental genetic studies on salmonids: morphogenesis, isozyme phenotypes and chromosomes in hybrid embryos. *Mem. Fac. Fish. Hokkaido Univ.* **31**(1–2): 1–94.

Arai, K. 1986. Effects of allotriploidization on development of hybrids between female chum salmon and male brook trout. *Bull. Jpn. Soc. Sci. Fish.* **52**(5): 823–829.

Axelrod, R., and W. D. Hamilton. 1981. The evolution of cooperation. *Science* **211:** 1390–1396.

Benfey, T. J. 1991. The physiology of triploid salmonids in relation to aquaculture. *Can. Tech. Rep. of Fish. Aquat. Sci.* **1789:** 73–80.

Benfey, T. J., and A. M. Sutterlin. 1984. Growth and gonadal development in triploid landlocked Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **41:** 1387–1392.

Benfey, T. J., I. I. Solar, G. de Jong, and E. M. Donaldson. 1986. Flow-cytometric confirmation of aneuploidy in sperm from triploid rainbow trout. *Trans. Am. Fish. Soc.* **115:** 838–840.

Beaumont, A., and J. Fairbrother. 1991. Ploidy manipulation in molluscan shellfish: a review. *J. Shellfish Res.* **10**(1): 1–18.

Bogart, J. P. 1980. Evolutionary significance of polyploidy in amphibians and reptiles. Pp. 341–378 in *Polyploidy, Biological Relevance,* W. H. Lewis, ed. Plenum Press, New York.

Breese, W. P., and R. E. Malouf. 1975. *Hatchery Manual for the Pacific Oyster.* Oregon State University Sea Grant College Program, Publication no. ORESU-H-75002.

Cauderon, Y. 1986. Cytogenetics in breeding programs dealing with polyploidy, interspecific hybridization and introgression. Pp. 83–104

in *Genetic Manipulation in Plant Breeding*, W. Horn *et al.*, eds. Walter de Gruyter, New York.

Chevassus, B., R. Guyomard, D. Chourrout, and E. Quillet. 1983. Production of viable hybrids in salmonids by triploidization. *Genet. Sel. Evol.* **15**: 519–532.

Cimino, M. C. 1973. Meiosis in triploid all-female fish (*Poeciliopsis*, Poeciliidae). *Science* **175**: 1484.

deWet, J. M. J. 1980. Origins of polyploids. Pp. 3–15 in *Polyploidy, Biological Relevance*, W. H. Lewis, ed. Plenum Press, New York.

Eichenlaub-Ritter, U., and I. Boll. 1989. Nocodazole sensitivity, age-related aneuploidy, and alterations in the cell cycle during maturation of mouse oocytes. *Cytogenet. Cell Genet.* **52**: 170–176.

Fankhauser, G. 1945. The effects of changes in chromosome number on amphibian development. *Q. Rev. Biol.* **20**: 20–78.

Fankhauser, G., and R. R. Humphrey. 1950. Chromosome number and development of progeny of triploid axolotl females mated with diploid males. *J. Exp. Zool.* **116**: 207–240.

Fankhauser, G., and R. R. Humphrey. 1954. Chromosome number and development of progeny of triploid axolotl males crosses with diploid females. *J. Exp. Zool.* **126**: 33–58.

Gaulden, M. E. 1989. A model that explains the varying frequency of aneuploid children with maternal age (J-shaped curve) as well as aneuploidy of paternal origin. Pp. 253–257 in *Mechanism of Chromosome Distribution and Aneuploidy*, M. A. Resnick and B. K. Vig, eds. Liss, New York.

Gaulden, M. E. 1992. Maternal age effect: the enigma of Down syndrome and other trisomic condition. *Mutation Res.* **296**: 69–88.

Gui, J. F., W. H. Xiao, L. Chen, S. C. Liang, and Y. G. Jiang. 1991. Gonadal development in artificial triploid transparent colored crucian carp. *ACTA Zool. Sinica* **37**: 297–304.

Guo, X. 1991. *Studies on Tetraploid Induction in the Pacific Oyster, Crassostrea gigas (Thunberg).* Ph.D. Dissertation, University of Washington, Seattle, Washington.

Guo, X., and S. K. Allen, Jr. 1994a. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body 1 in eggs from triploids. *Mol. Mar. Biol. Biotechnol.* **3**(1): 42–50.

Guo, X., and S. K. Allen, Jr. 1994b. Sex determination and polyploid gigantism in the dwarf surf-clam (*Mulinia lateralis* Say). *Genetics* 138:1–8.

Guo, X., W. K. Hershberger, K. Cooper, and K. K. Chew. 1992. Genetic consequences of blocking polar body 1 with cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*. II. Segregation of chromosomes. *Biol. Bull.* **183**: 387–393.

Guo, X., W. K. Hershberger, K. Cooper, and K. K. Chew. 1993. Artificial gynogenesis with ultraviolet light-irradiated sperm in the Pacific oyster, *Crassostrea gigas*. I. Induction and survival. *Aquaculture* **113**: 201–214.

Hebert, P. D. N., and M. J. Beaton. 1989. *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis.* Helena Lab, Beaumont, Texas. 32 pp.

Hecht, F., and B. K. Hecht. 1987. Aneuploidy in human: dimensions, demography, and dangers of abnormal numbers of chromosomes. Pp. 9–49 in *Aneuploidy, Part A: Incidence and Etiology*, B. K. Vig and A. A. Sandberg, eds. Alan R. Liss, Inc., New York.

Jones, R. E., and R. Bamford. 1942. Chromosome number in the progeny of triploid Gladiolus with special reference to the contribution of the triploid. *Am. J. Bot.* **29**: 807–813.

Kloss, R. J., and R. M. Nesse. 1992. Trisomy: chromosome competition or maternal stragegy. *Ethol. Sociobiol.* **13**: 283–287.

Kratzer, P. G., M. S. Golbus, S. A. Schonberg, D. C. Heilbron, and R. N. Taylor. 1992. Cytogenetic evidence for enhanced selective miscarriage of trisomy 21 pregnancies with advancing maternal age. *Am. J. Med. Genet.* **44**: 657–663.

Lincoln, R. F. 1981a. Sexual maturation in triploid male plaice (*Pleuronectes platessa*) and plaice × flounder (*Platichthys flesus*) hybrids. *J. Fish Biol.* **19**: 415–426.

Lincoln, R. F. 1981b. Sexual maturation in female triploid plaice, *Pleuronectes platessa*, and plaice × flounder, *Platichthys flesus*, hybrids. *J. Fish Biol.* **19**: 499–507.

Lincoln, R. F., and A. P. Scott. 1984. Sexual maturation in triploid rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **25**: 385–392.

Lu, J.-K. 1986. *The Combined Effects of Salinity and Temperature on Meiosis and Early Mitosis of the Pacific Oyster* (Crassostrea gigas) *Oocytes. M.S. Thesis, University of Washington, Seattle.*

Mailhes, J. B. 1987. Incidence of aneuploidy in rodents. Pp. 67–101 in *Aneuploidy, Part A: Incidence and Etiology*, B. K. Vig and A. A. Sandberg, eds. Alan R. Liss, Inc., New York.

Morgan, L. V. 1925. Polyploidy in *Drosophila melanogaster* with two attached X chromosomes. *Genetics* **10**: 148–178.

Myers, W. M. 1944. Cytological studies of a triploid perennial rye grass and its progeny. *J. Hered.* **35**: 17–23.

Parsons, J. E., R. A. Busch, G. H. Thorgaard, and P. Scheerer. 1986. Increased resistance of triploid rainbow trout × coho salmon hybrids to infectious hematopoeitic necrosis virus. *Aquaculture* **57**: 337–343.

Pellestor, F. 1991. Differential distribution of aneuploidy in human gametes according to their sex. *Human Reprod.* **6**(9): 1252–1258.

Punyasingh, K. 1947. Chromosome number in crosses of diploid, triploid and tetraploid maize. *Genetics* **32**: 541–554.

Quayle, D. B. 1988. Pacific oyster culture in British Columbia. *Can. Bull. Fish. Aquat. Sci.* **218**: 1–241.

Scheerer, P. D., and G. H. Thorgaard. 1983. Increased survival in salmonid hybrids by induced triploidy. *Can. J. Fish. Aquatic Sci.* **40**: 2040–2044.

Schultz, R. J. 1980. Role of polyploidy in the evolution of fishes. Pp. 313–340 in *Polyploidy, Biological Relevance*, W. H. Lewis, ed. Plenum Press, New York.

Takaesu, N., P. A. Jacobs, A. Cockwell, and R. D. Blackston. 1990. Nondisjunction of chromosome 21. *Am. J. Med. Genet.* Suppl. **7**: 175–181.

Thorgaard, G. H. 1983. Chromosome set manipulation and sex control in fish. Pp. 405–434 in *Fish Physiology*, vol. 9(B), W. S. Hoar, D. J. Randall, and E. M. Donaldson, eds. Academic Press, London.

Thorgaard, G. H. 1986. Ploidy manipulation and performance. *Aquaculture* **57**: 57–64.

Thorgaard, G. H., and G. A. E. Gall. 1979. Adult triploids in a rainbow trout family. *Genetics* **93**: 961–973.

Warburton, D. 1989. The effect of maternal age on the frequency of trisomy: change in meiosis or in utero selection? Pp. 165–181 in *Molecular and Cytogenetic Studies of Nondisjunction*, T. J. Hassold and C. J. Epstein, eds. Liss, New York.

White, M. J. D. 1978. *Modes of Speciation.* W. H. Freeman and Company, San Francisco. Pp. 261–285.

Wilson, G. B. 1946. Cytological studies in Musae. I. Meiosis in some triploid clones. *Genetics* **31**: 241–258.

Yamano, K., E. Yamaha, and F. Yamazaki. 1988. Increased viability of allotriploid pink salmon × Japanese char hybrids. *Nippon Suisan Gakkaishi* **54**(9): 1477–1481.

Zenzes, M. T., and R. F. Casper. 1992. Cytogenetics of human oocytes, zygotes, and embryos after in vitro fertilization. *Human Genet.* **88**: 367–375.

Zheng, C. J., and B. Byers. 1992. Oocyte selection: a new model for the maternal-age dependence of Down syndrome. *Human Genet.* **90**: 1–6.